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Opinion

The future of biological warfare

Arturo Casadevall*
Department of Microbiology and Immunology and the Division of Infectious Diseases of the Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY, USA.

It is an axiom of human history that whatever technology is available will be applied in warfare as one side or the other seeks to gain an advantage. Humans are unique among the species in their capacity for fighting prolonged conflicts where the nature of the war reflects the types of technologies available. Stone, metal, leather, wood, domesticated animals, wheels, etc. were each exploited by ancient societies in warfare. In late antiquity the adoption of the stirrup in Western Europe transformed warfare by enhancing the fighting capacity of the mounted warrior, which eventually led to the emergence and prominence of the knightly class. More recently gunpowder, steam engines, aircraft, chemicals, electronics and nuclear physics were employed in warfare. In each epoch, the technologies available had enormous influence on the strategy and tactics used. Biological warfare is ancient but its applicability to the battlefield has been limited by its unpredictability, blowback possibility and uncertain efficacy. However, the biological revolution that began in the mid-20th century has led to the development of powerful technologies that could potentially be used to generate new biological weapons of tremendous destructive power. Although biological warfare is currently prohibited by the 1972 Biological and Toxic Weapons Convention (BTWC) a review of prior attempts to limit the use of certain weapons such as the medieval crossbow, and more recently gas warfare, provides little encouragement for the notion that a technology that is useful in war can be limited by treaty. Furthermore, the BTWC restrictions apply only to signatory nation states and are irrelevant to terrorist organizations or lone wolves type of terrorists. Given the human track record for conflict and the potential power of biological warfare we are led to the sad conclusion that biological warfare has a future, and that society must prepare for the eventuality that it will used again by either nations or individuals. In this essay I will try to peek into the far horizon to identify some general themes that might be helpful in protecting against future horrors fully aware that the nature of technological change is so rapid and profound that any such view must necessarily be myopic.

Received 13 October, 2011; revised 3 January, 2012; accepted 22 February, 2012. *For correspondence. E-mail Arturo.casadevall@einstein.yu.edu; Tel. (+1) 718 430 3665; Fax (+1) 718 430 8711.

Existential threats to humanity

In considering the importance of biological warfare as a subject for concern it is worthwhile to review the known existential threats. At this time this writer can identify at three major existential threats to humanity: (i) large-scale thermonuclear war followed by a nuclear winter, (ii) a planet killing asteroid impact and (iii) infectious disease. To this trio might be added climate change making the planet uninhabitable. Of the three existential threats the first is deduced from the inferred cataclysmic effects of nuclear war. For the second there is geological evidence for the association of asteroid impacts with massive extinction (Alvarez, 1987). As to an existential threat from microbes recent decades have provided unequivocal evidence for the ability of certain pathogens to cause the extinction of entire species. Although infectious disease has traditionally not been associated with extinction this view has changed by the finding that a single chytrid fungus was responsible for the extinction of numerous amphibian species (Daszak *et al.*, 1999; Mendelson *et al.*, 2006). Previously, the view that infectious diseases were not a cause of extinction was predicated on the notion that many pathogens required their hosts and that some proportion of the host population was naturally resistant. However, that calculation does not apply to microbes that are acquired directly from the environment and have no need for a host, such as the majority of fungal pathogens. For those types of host–microbe interactions it is possible for the pathogen to kill off every last member of a species without harm to itself, since it would return to its natural habitat upon killing its last host. Hence, from the viewpoint of existential threats environmental microbes could potentially pose a much greater threat to humanity than the known pathogenic microbes, which number somewhere near 1500 species (Cleaveland *et al.*, 2001; Taylor *et al.*,

2001), especially if some of these species acquired the capacity for pathogenicity as a consequence of natural evolution or bioengineering.

The universe of threats

The universe of threats can potentially encompass all microbes that inhabit the planet. Although most authorities divide microbes into those that are pathogenic and non-pathogenic there is a fundamental fallacy in assigning the property of pathogenicity to a microbe alone, for virulence is a microbial property that is expressed only in a susceptible host (Casadevall and Pirofski, 2001). For example, highly virulent microbes such as variola major virus are not virulent in hosts immunized with vaccinia. On the other hand, microbes normally avirulent for immunologically competent hosts such as *Aspergillus* spp. can be highly pathogenic for hosts with impaired immunity. The fact that virulence is expressed only in a susceptible host implies that it is not an independent microbial property. This is an important concept for it makes it difficult to unequivocally exclude any particular microbe as a potential threat.

Given the enormous microbial diversity in the planet it is remarkable that there are relative few microbes capable of causing human disease. This paucity presumably reflects the effectiveness of vertebrate immunity combined with high temperatures that exclude the overwhelming majority of environmental microbes (Casadevall and Pirofski, 2007; Robert and Casadevall, 2009). Pathogenic microbes can be divided into two general groups, those acquired from other host and those acquired from the environment. Pathogenic microbes acquired from other hosts tend to be host adapted, are relatively few in number, and include most of the well-known pathogens. Host-acquired pathogenic microbes are usually communicable and have historically been responsible for devastating epidemics. In contrast, pathogenic microbes acquired directly from the environment represent a completely different challenge for the host since these have acquired their capacity for pathogenicity by virtue of non-mammalian selection pressures, such as the interaction with amoebae (Casadevall and Pirofski, 2007).

Among environmental microbes the major threats to humans come from those microbes that can survive mammalian temperatures. Although the elevated temperatures of mammals almost certainly create a thermal exclusionary environment for a large percentage of environmental microbes, one cannot automatically dismiss microbes that are not thermal tolerant. In this regard it is noteworthy that it was possible to adapt an insect pathogenic fungus to tolerate mammalian temperatures by simple thermal selection in the laboratory (de Crecy *et al.*, 2009). Whether this adaptation conferred the capacity for mammalian virulence is unknown but the example provides a precedent for the notion that it may be possible to greatly enlarge the number of microbes with human pathogenic capacity by simple selection for more thermally stable variants.

If the universe of threats from the natural world was not enough humanity also faces potential threats from synthetic biology (Tucker and Zilinkas, 2006; Tucker, 2011). Although the risk of generating Frankenstein microbes accidentally from synthetic biology is quite low, it is not zero. Given sufficient time, experimentation and selection it is possible that technologies emerging from synthetic biology-related research can find applications in biological warfare.

Preparing against the known and unknown

Despite a universe of threats that is overwhelming with regards to the number of microbes with pathogenic potential current biodefence efforts remain focused on a tiny proportion of biological threats. In fact, governments have responded to the threat of bioterrorism by the creation of lists that aim to protect society by restricting access to certain microbes and toxins and creating legal tools for the prosecution of individuals on the basis of possession alone (Casadevall and Relman, 2010). Furthermore, such lists have been used to prioritize the development of countermeasures such as increased vigilance, detection devices, diagnostics, vaccines, drugs and therapeutic immunoglobulins. In general, microbial threat lists have been designed by creating algorithms that attempt to identify the most dangerous types of microbes. Although such algorithms are not in the public domain some hint of the types of considerations taken into account in the generation of such lists can be found in an article authored by scientists from the Center of Diseases of Control (Atlanta, GA) (Rotz *et al.*, 2002), the institution responsible for the administering the Select Agent and toxins regulations. It is noteworthy that their risk matrix analysis for assessing the public health impact of potential biological terrorism agents included such diverse criteria as mortality, need for hospitalization, likelihood for dissemination, availability of countermeasures and public perception (Rotz *et al.*, 2002). The last parameter is interesting since public recognition of a known danger such as anthrax spores is far more likely to cause panic and societal disruption than less well-known threats.

A fundamental problem with any microbial threat list is that it is necessarily a backward looking document. History consistently shows that generals always prepare to fight the last war and biological warfare is probably no exception. In this regard, microbial threat lists are primarily populated with agents that have been investigated by the military for biological warfare use, such as *Bacillus anthracis*, or have caused terrible epidemics in history,

such as variola major and *Yersinia pestis*. Organisms like fungi that have not been associated with major epidemics tend to be ignored in threat analysis scenarios despite the fact that this kingdom, as a whole, includes many species with high weapon potential (Casadevall and Pirofski, 2006) and the fact that fungal diseases are currently decimating certain amphibian and bat populations. Moreover, recent decades have seen the emergence of numerous new microbial diseases including the human immunodeficiency virus (HIV), severe acute respiratory syndrome (SARS) coronavirus, Ebola virus, *Legionella* spp., etc. At least 335 new infectious diseases have been described since 1940, with the majority being zoonosis (Jones *et al*., 2008). The identification of so many new diseases over the past seven decades shows no sign to slowing, and it is almost a certainty that humanity will continue to confront new microbial threats and that many of these agents, such as SARS coronavirus, possess a significant weapon potential when recovered from nature (Casadevall and Pirofski, 2004). However, the experience with SARS in 2003 also provides encouragement that even the emergence of a new agent that disseminates rapidly worldwide can be contained. In that outbreak international cooperation combined with good surveillance and a healthy research environment that was able to rapidly identify the agent within weeks of the outbreak, generate diagnostic methods and produce a therapeutic mAb in about 1 year. Consequently biodefence efforts are intimately linked to surveillance efforts for emerging infectious diseases and any defence strategy against biological weapons much consider the development of countermeasures against yet identified threats.

The near and far horizons

The realization that a handful of envelopes containing *B. anthracis* in 2001 was sufficient to cause widespread panic, and precipitated the first evacuation of the houses of the US government since the war of 1812, provided a clear demonstration of the power of cheap biological weapons. In an age of terrorism biological weapons are perfectly suited for asymmetric warfare where the relatively low costs of producing such weapons combined with their potential for amplification through communicability have a disproportionately strong effect on targeted populations. Consequently, biological weapons are likely to remain very attractive to terrorists and fringe groups like millennial sects. Thus the near horizon is likely to witness continued concern about low intensity use of biological weapons fashioned around known pathogenic microbes such as *Salmonella* spp. and *B. anthracis*, which have already been used in terrorism.

The scene on the far horizon is much harder to discern simply because the current rapid the pace of technological advance suggests that new technologies are likely to be developed in coming years that will completely change the landscape for biological warfare offensive and defensive possibilities. Even without envisioning new biological agents, such as those that could be generated by synthetic biology, the technology already exists for significantly enhancing the lethality of biological weapons. The introduction of antimicrobial resistance genes into bacterial agents could significantly enhance their lethality by reducing treatment options. In this regard, it is relatively easy to generate *B. anthracis* resistant to first line antimicrobial therapies such as ciprofloxacin (Athamna *et al*., 2004). The efficacy of vaccines can be circumvented by genetically modifying agents to express immune modifier genes that interfere with the immune response as was demonstrated by the expression of IL-4 in ectromelia virus (Jackson *et al*., 2001). It is noteworthy that microbial modifications to increase lethality is only one possible outcome for engineering biological weapons since these could also be designed to incapacitate instead of kill.

Given the enormous universe of microbial threats, the power of modern biology to enhance the microbial virulence and the high likelihood that biological weapons will continue to threaten humanity one must face the question of how best to protect society. The sheer number of threats and the availability of technologies to modify microbes to defeat available countermeasures suggest that any attempt to achieve defence in depth using microbe-by-microbe approaches to biodefence is impractical and ineffective.

A prescription for defence in depth

i. Continued development of specific diagnostic assays and countermeasures (vaccines, drugs, antibodies) for high risk threats identified by current matrix threat analysis. This is essentially a continuation of the major societal response to perceived biological threats in the first decade of the 21st century when a significant proportion of government supported research has focused on known agents such as variola major, *B. anthracis* and other high risk agents. This approach makes sense given that known agents will continue to be the most likely threats in the near horizon.
ii. Develop host-targeted interventions that enhance immune function against a wide variety of threats. In other words, develop therapies that produce temporary increases in immune function that would protect against known and unknown threats. This approach would provide defensive options against yet to be identified microbial threats.
iii. Develop new ways to assess the healthy state that could allow monitoring of the population to identify the

appearance of new agents. Although physicians can readily identify the disease state and surveillance systems for known agents are critically important for identifying a biological attack, such approaches may not suffice for all threats. For example, consider the situation with the outbreak of the HIV epidemic. The epidemic was identified in 1981 as a consequence of clusters of cases with known infectious diseases that did not fit known epidemiological parameters for such maladies as they included rare diseases in individuals with no predisposing conditions. However, we now know that AIDS can follow many years after the HIV infection and the interval between infection and disease is characterized by a slow decline in immune function during which the individual does not exhibit signs of disease. Arguably, the existence of methodology that could assess the healthy state might have identified the silent spread of the virus in certain populations years prior to the onset of the epidemic.

iv. Obtain a better understanding of microbial diseases in animal species and especially those that come in close contact with humans. Given that 72% of emergent infectious diseases described in recent decades have been zoonosis (Jones *et al*., 2008), it is reasonable to assume that wildlife will continue to be source of new pathogenic microbes for humans and a potential source of biological weapons. Consequently any effort to design a system for defence in depth should include efforts to describe, catalogue and study microbial diseases in wildlife.

v. In preparing for known and unknown threats the availability of a vigorous scientific research establishment that can respond rapidly is an essential component for any effort to defend society. The rapid identification of HIV as the cause of AIDS and the development of effective anti-retroviral therapies was made possible by prior societal investments in studying the biology of retroviruses at a time when these were not associated with human diseases. Hence, continued investments in basic research with emphasis on fostering a better understanding of host–microbe interactions is an essential cornerstone for any effort to defend in depth against biological weapons.

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